THE MITOTIC INDEX OF THE PALMAR AND PLANTAR EPIDERMIS IN RESPONSE TO STIMULATION

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In 1927 Hoepke (4) wrote an excellent account on epidermal regeneration together with an exhaustive bibliography. During the same year there appeared the first volume of Jadassohn's handbook of skin and venereal diseases, *Anatomy of the Skin* by Pinkus (6). The latter was emphatic in voicing his opinion that "mitotic division in the basal cell layer is the only normal mode of cell division" thus supporting and restricting even the view held by Flemming (2) sixty years ago.

In previous studies on epidermal regeneration of the scalp (Thuringer (7, 8)) the results obtained were out of line with the accepted notion that mitosis takes place principally in the basal cell layer, and that very few mitotic figures are found in the adjacent layers of spinous cells. Measured areas of epidermis of the scalp obtained from executions, for example, showed the distribution of mitotic figures, basal cell layer 12 per cent; lower third of spinous cell layer 30 per cent; middle third of spinous cell layer 46 per cent, and outer third of spinous cell layer 12 per cent.

Quantitatively there was an average of 10 mitoses present to every square millimeter of epidermis. Attention was called to the occurrence of growth waves, i.e., areas in the epidermis in which a larger number of mitoses were found alternating with areas in which they were extremely scarce or completely missing, thus suggesting a rhythmic cell production. The data obtained were not to be regarded as criteria for epidermal surfaces other than that of the scalp.

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Opportunities presented themselves from time to time to study perfectly normal skin from other body regions, but it was not until four years later that a chance was afforded to study the epidermis of the normal prepuce of a 17-day and a 3-year-old child. In this work it was observed that the distribution of mitoses varied from those previously mentioned. The existence of a regenerative or mitotic rhythm was confirmed and a **fixation-time factor** as well as **regional** and **age-growth factors** were recognized. The time for the completion of mitosis was determined at about 15 to 30 minutes, which is about one-half the time previously estimated for this process. (Lewis and Lewis (5)).

The **fixation-time factor,** determined by noting the ratio of mitoses to resting cells at various intervals after removal of the prepuce was as follows: for the 17-day material instant fixation, 1:178.2; five minutes later, 1:273.1; ten minutes later, 1:418.5 and fifteen minutes after removal 1:633.8. This information should be of practical importance to anyone interested in the study of the relative malignancy of tumors since some writers (Evans (1)) disclaim such a factor entirely.

The age-growth factor may be appreciated by comparing the figures given above from material of the 17-day-old child to that obtained from a boy of three years; instant fixation 1:1359; five minutes delay 1:5120, and fifteen minutes delay 1:11,814.

The differences of epidermal regeneration due to the existence of a **regional factor** have received practically no attention. Most of the recorded observations were made upon skin obtained from the palmar or plantar surfaces, yet the impression was conveyed that the conditions found there were identical to those in other parts of the epidermis, until the figures just quoted proved otherwise. It is only reasonable to surmise that those parts of the epidermis constantly exposed to pressure, friction, or great thermal changes must necessarily regenerate at a more rapid rate to compensate for wear and tear than does the general epidermis which is not subject to such environmental factors.

Since all these observations cited were made on normal "resting" epidermis, it was but natural to proceed a step and attempt to determine the mitotic index of an epidermal surface in response to stimulation. With the exception of Garten's (1895 (3)) classic experiment, the literature did not reveal any information bearing on the subject. Garten made a very ingenious device which he strapped to the calf of his leg. It consisted of a small clockwork operating a spring which exerted a gentle blow upon the skin at intervals of one minute. After 66 hours he excised a small piece of skin from this area and drew the conclusion that the epidermis had slightly increased in thickness due to better nourishment of its cells induced by the alternate pressure and relaxation.

It was decided to study the behavior of the palmar and plantar epidermis, since no previous quantitative data was available. This highly specialized region represents only about $\frac{1}{20}$ to $\frac{1}{22}$ of of the entire epidermal surface. It is in this region that most of the earlier investigators noted that mitoses were found chiefly in the basal cell layer, with occasional ones in the one or two adjacent layers. Practically all textbook illustrations showing detail of the epidermis were made from sections of palmar or plantar surfaces, "on account of the facility with which the various layers of cells could be identified." After a few very painful attempts to contribute skin as well as information, the idea of using human epidermis was abandoned and search made for a suitable substitute.

MATERIALS AND METHODS

The palmar and plantar pads of cats proved ideal for this work since they resemble the human palmar and plantar epidermis both functionally and morphologically, even to the thickness and number of cells per layer. Only specimens with unpigmented pads were selected. In all but one instance cell counts were made of the resting epidermis, i.e., prior to stimulation. The manner of stimulation was electrical and mechanical, the latter being carried out first by walking the animal tied to a leash, and second by placing the animal in a revolving drum four feet in diameter. Great care had to be exercised to avoid exciting the animals, since a number of preliminary experiments showed that data obtained from a frightened animal did not give uniform results.

A piece of celluloid provided with a suitable small oval opening was pressed over the selected area and the projecting skin cut with an oblique stroke of a safety razor blade. A little experience produced sections of skin only slightly thicker than the epidermal layer, and apparently without appreciable discomfort to the animal. All material was instantly fixed in Zenker's fluid, the tissues were embedded by the combined celloidin-paraffin technique and cut in serial sections of 10 micra in thickness. Mann's hemotoxylin and eosin were used for staining.

In conformity with previous work and to facilitate the counting of dividing cells, the stratum germinativum was considered in four divisions; namely, the stratum basale and the stratum spinosum, which was arbitrarily divided into lower, middle, and upper one-third regions to form the remaining three groups.

An actual count was made of all cells in the sections and of all the mitoses. Furthermore, for the purpose of obtaining complete data on the regenerative processes and to corroborate the previously recorded time for cell division, the phase of each mitotic figure was noted, including the axes of division with respect to the horizontal plane.

To obtain data on normal or resting stage material, the cats were fed in the evening and permitted the run of their quarters for two hours. They were then confined to clean, comfortable cages in individual rooms. Early the next day the animals were removed from their cages and sections of epidermis taken at once.

EXPERIMENTAL

Experiments 1 to 5. The palmar and plantar pads of five cats were used to establish the ratio of mitosis in normal resting epidermis. In fifty sections, 210,765 cells were counted including 500 mitoses which were distributed as follows: str. basale 44.5 per cent; str. spinosum, lower $\frac{1}{3}$, 54.5 per cent; middle $\frac{1}{3}$, 1 per cent; upper $\frac{1}{3}$, 0 per cent.

Experiment 6. Mechanical stimulation by walking. The rested animal was leashed and a small piece of palmar pad removed at once. A second piece of tissue was taken after a walk of half an hour. This animal was excited from the beginning of the experiment, went into convulsions later and was killed.

Table 1, (a, b) and summary visualize the cell counts of the resting and stimulated epidermis together with their mitotic index. The totals under (a) are within 2 per cent of the averages

TABLE 1

LOCATION	PROPHASE	METAPHASE	ANAPHASE	TELOPHASE	TOTAL
(a) Mitotic figures of rest material. 19 sections; 65,018 cells					
Stratum spinosum					
Upper $\frac{1}{3}$	0	0	0	0	0
Middle $\frac{1}{3}$	1	0	0	0	1
Lower $\frac{1}{3}$	23	15	2	4	44
Str. basale	17	8	6	2	33
Total	41	23	8	6	78
(b) Mitotic figures of stimulated material (walking). 9 sections; 58,878 cells					
Stratum spinosum					
Upper $\frac{1}{3}$	0	0	0	0	0
Middle $\frac{1}{3}$	2	1	0	0	3
Lower $\frac{1}{3}$	13	6	0	0	19
Str. basale	28	1	1	2	32
Total	43	8	1	2	54

Summary

On the basis of 100,000 cells

LOCATION	REST	STIMULATION	
Stratum spinosum			
Upper $\frac{1}{3}$	0	0	
Middle $\frac{1}{3}$	1	4	46% decrease
Lower $\frac{1}{3}$	67	33	
Str. basale	50	62	20% increase
Total	118	99	18% decrease
Ratio	1:866	1:1,090	

cited above, while (b) represents not only a sharp reversal in the activity of the basal and spinous cell layers but actually an acute drop in the number of mitoses in the latter.

Experiment 7. Electrical stimulation. A small cage was provided with a wooden floor board having a series of closely spaced wires leading to opposite poles of an inductorium. A control piece of epidermis was taken at the resting stage. A mild shock

TABLE 2

LOCATION	PROPHASE	METAPHASE	ANAPHASE	TELOPHASE	TOTAL
(a) Mitotic figures of rest material. 12 sections; 31,128 cells					
Stratum spinosum					
Upper ⅓	1	0	0	0	1
Middle ⅓	1	1	0	0	2
Lower ⅓	31	6	7	6	50
Str. basale	42	17	15	6	80
Total	75	24	22	12	133
(b) One-half hour electrical stimulation. 10 sections; 37,280 cells					
Stratum spinosum					
Upper ⅓	0	0	0	0	0
Middle ⅓	4	1	2	1	8
Lower ⅓	72	16	9	5	102
Str. basale	93	17	10	3	123
Total	169	34	21	9	233

Summary

On the basis of 100,000 cells

LOCATION	REST	STIMULATION	
Stratum spinosum			
Upper ⅓	2.5	0	
Middle ⅓	7.5	22	75% increase
Lower ⅓	160	275	
Str. basale	258	334	29.1% increase
Total	428	631	46% increase
Ratio	1:234	1:158	

was produced every five seconds and a second piece of tissue removed after half an hour. This animal was very docile and responded well to handling.

All preceding experiments furnished data on the mitotic index of the resting epidermis, and experiments 6 and 7 on the influence of one-half hour of mechanical and electrical stimulation. The following questions, however, remained unanswered: 1. In what manner does the epidermis respond to continuous natural stimulation, such as walking? 2. How much stimulation is required to produce an appreciable increase in mitoses, and when does it occur? 3. What is the nature of such a response with respect to its duration, amplitude and return to normal? 4. Is the reaction of the str. basale and str. spinosum identical?

Experiment 8. It was decided to stimulate the epidermis for several hours using the drum mentioned. To maintain the normal conditions as much as possible no tissue was removed at the beginning of the experiment. The drum was rotated slowly and reversed or stopped momentarily according to the actions of the cat. A rest of one minute was allowed every 15 minutes, and 5 minutes out of the wheel every hour to keep the animal contented. After $4\frac{3}{4}$ hours it showed signs of some discomfort and the experiment was terminated at 5 hours. A piece of tissue was then removed from the palmar pad of the left paw, and shortly thereafter the animal responded nicely to petting, purring quite contentedly. Pieces of tissue were removed from the other pads at hourly intervals. This animal recovered without ill effects.

An analysis of table 3 (parts a, b, and c) reveals at a glance that the height of the cellular response apparently had been reached prior to the end of the five hour stimulation period, since the drop of the mitotic index was constant in the tissues removed at zero hour, one hour, two hours, and three hours after stimulation.

This unexpected decline in the mitotic rate after 5 hours stimulation was the first tangible clue afforded in studying the regenerative capacity of the epidermis. Therefore the experiment was repeated and in order to follow the mitotic response, a piece of tissue was removed at the beginning of the experiment before placing the animal in the drum, and at hourly intervals during stimulation.

Experiment 9. Several months having passed, the same animal

TABLE 3

LOCATION	PROPHASE	METAPHASE	ANAPHASE	TELOPHASE	TOTAL
(a) At end of 5 hours stimulation. 20 sections; 110,208 cells					
Stratum spinosum					
Upper $\frac{1}{3}$	0	0	0	0	0
Middle $\frac{1}{3}$	1	0	0	0	1
Lower $\frac{1}{3}$	72	14	0	0	86
Str. basale	44	4	0	0	48
Total	117	18	0	0	135
(b) One hour later. 20 sections; 77,520 cells					
Stratum spinosum					
Upper $\frac{1}{3}$	0	0	0	0	0
Middle $\frac{1}{3}$	0	1	0	0	1
Lower $\frac{1}{3}$	20	7	3	2	32
Str. basale	22	3	1	1	27
Total	42	11	4	3	60
(c) Two hours later. 20 sections; 90,625 cells					
Stratum spinosum					
Upper $\frac{1}{3}$	0	0	0	0	0
Middle $\frac{1}{3}$	0	0	0	0	0
Lower $\frac{1}{3}$	3	3	3	0	9
Str. basale	8	1	0	0	9
Total	11	4	3	0	18
(d) Three hours later. 20 sections; 85,134 cells					
Stratum spinosum					
Upper $\frac{1}{3}$	0	0	0	0	0
Middle $\frac{1}{3}$	0	0	0	0	0
Lower $\frac{1}{3}$	3	0	1	4	8
Str. basale	6	4	4	2	16
Total	9	4	5	6	24

Summary

On the basis of 100,000 cells

	0 HOUR	1 HOUR	2 HOURS	3 HOURS
Str. spinosum	80	41	10	9
Str. basale	43	34	10	20
Total	123	75	20	29
Cells	100,000	100,000	100,000	100,000

TABLE 4

LOCATION	PROPHASE	METAPHASE	ANAPHASE	TELOPHASE	TOTAL
(a) Mitotic figures of rest material. 8 sections; 30,350 cells					
Stratum spinosum					
Upper ⅓	0	0	0	0	0
Middle ⅓	1	0	0	0	1
Lower ⅓	29	16	9	1	55
Str. basale	25	7	4	0	36
Total	55	23	13	1	92
(b) One hour stimulation. 5 sections; 21,875 cells					
Stratum spinosum					
Upper ⅓	0	0	0	0	0
Middle ⅓	4	2	0	0	6
Lower ⅓	70	74	5	0	149
Str. basale	46	16	2	1	65
Total	120	92	7	1	220
(c) Two hours stimulation. 5 sections; 16,660 cells					
Stratum spinosum					
Upper ⅓	0	0	0	0	0
Middle ⅓	1	0	1	0	2
Lower ⅓	78	79	9	1	167
Str. basale	18	16	0	0	34
Total	97	95	10	1	203
(d) Three hours stimulation. 5 sections; 22,410 cells					
Stratum spinosum					
Upper ⅓	0	0	0	0	0
Middle ⅓	2	1	0	0	3
Lower ⅓	59	71	11	3	144
Str. basale	16	6	0	0	22
Total	77	78	11	3	169

Summary

On the basis of 100,000 cells. 23 sections; 120,000 cells

	0 HOUR	1 HOUR	2 HOURS	3 HOURS
Upper ⅓	0	0	0	0
Middle ⅓	3	26	10	13
Lower ⅓	183	676	982	653
Str. basale	120	298	200	100
Total	306	1000	1192	766
Ratio	1:326	1:100	1:83	1:130

was used; all pads were in perfect condition. Shortly after the fourth piece of tissue was removed the animal began to vomit and the experiment terminated at 3 hours. Following removal from the cage it seemingly returned to normal condition; however, the following morning it was found dead in its cage.

DISCUSSION

The first five experiments showed the mitotic index of the normal resting palmar-plantar epidermis as found in the palmar and plantar pads of the cat. The average number of mitoses present in the resting epidermis were distributed as follows: Stratum basale 44.5 per cent, stratum spinosum, lower $\frac{1}{3}$, 54.5 per cent; middle $\frac{1}{3}$, 1 per cent and in the upper $\frac{1}{3}$, 0 per cent.

The accepted view that most of the mitoses in the palmar and plantar epidermis are found in the lower part of the stratum germinativum is quite true. Cursory examination of individual sections without detailed total cell counts give the impression of the prevalence of mitoses in the basal cell layer. On account of the uniform axes of division and arrangement of the cells in the basal cell layer, mitotic figures here stand out in sharp contrast; this is not always the case in the spinous cell layer where the cells are frequently cut in such manner as to make mitotic figures less conspicuous.

The figures obtained in the five experiments mentioned were substantiated by those found in the resting epidermis in experiment 9 (resting stage). No reason can be given for the increased activity shown by the basal cell layer over the lower one-third of the spinous cell layer.

The results of experiment 6 (a and b) are contradictory. The number of mitoses present at the beginning of the experiment were about one-half of those found in other "resting epidermis"; furthermore, the increase upon stimulation was negligible and apparently caused by some abnormal factor. The unusually frightened condition of the animal was thought responsible for the peculiar cellular behavior, rather than any direct detrimental action resulting from stimulation.

Any alteration of the nutritional state of the epidermis, either in quantity or quality would first affect the cells more remote from the source of supply; i.e., the stratum spinosum. Likewise a diminution in strength of the so-called trophic impulses would first inhibit the more peripheral cells. Other experiments showed that thirty minutes of mild stimulation did not lead to a decline in the number of mitoses in the stratum spinosum. The attempted explanation is a conjecture based upon general knowledge of the nutritional and nervous influences on cellular life and activity.

In experiment 7 (resting epidermis) the stratum basale was more active than the stratum spinosum; 80 mitoses in the former to 50 mitoses in the latter. After half an hour of electrical stimulation the lower $\frac{1}{3}$ of the stratum spinosum responded with an increase in mitoses almost equal to that observed in the stratum basale; namely, 110 to 123.

Experiments 8 and 9 confirmed the first six experiments and supplied information about the mitotic rate of the normal unstimulated epidermis, as well as furnishing answers to the questions enumerated before. The activity of the lower $\frac{1}{3}$ of the spinous cell layer was almost in a ratio of 2 to 1 of the basal cell layer.

On the basis of the material contained in summaries to tables 3 and 4, two graphs were constructed. Graph 1 based on experiment 8 indicates a steep decline in the mitotic rate over a three hour period from the cessation of stimulation. The total mitoses diminished from 123 on cessation of stimulation to 75 one hour later, and 20, two hours later. A rise to 29 was observed at the end of the third hour (Summary table 3, uniform cell count). It is of particular interest to follow the figures giving the data of the stratum basale and stratum spinosum independently.

Graph 2 of experiment 9 proved the assumption correct that the peak of regenerative activity had been reached before the end of the five hour stimulation period.

In reproducing the data of experiments 8 and 9 in the form of a combined graph (fig. 1) their position with regard to right and left was deliberately transposed. The top double line indicates the total mitoses counted, the broken line those of the stratum spinosum and the lower solid line those of the stratum basale.

Prior to the experiment 8, there was no information available for the rate of mitoses during stimulation; therefore, it was arbitrarily continued for five hours. At the end of that period 123 mitoses were found. One and two hours later this count

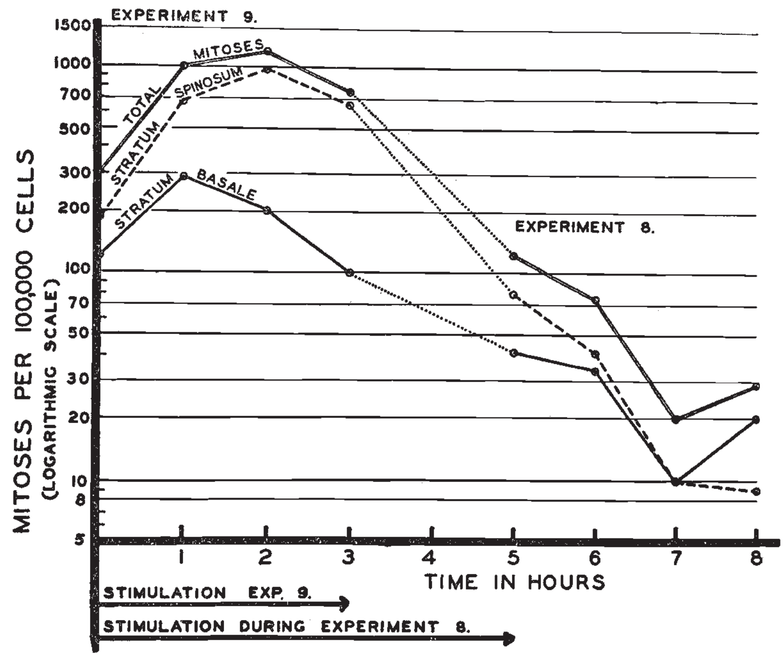

Fig. 1

was reduced to 75 and 20 mitoses respectively; after another hour showing an increase to 29 mitoses. These figures made evident that the peak of mitotic activity had been passed between the beginning and the fifth hour of stimulation.

The graph on the left side (from summary of experiment 9) shows the presence of 306 mitoses at the beginning of stimulation. This number increased to 1000 and 1192 during the first and second hours of stimulation, afterward showing a decline to 766 mitoses in spite of the continued stimulation. As stated before, the experiment had to be stopped in three hours. Consequently an interval appears in the graphs denoting the course of the mitotic rate had it been possible to continue this experiment for five hours. The direction of the dotted lines connecting the two graphs denote the probable decline in the mitotic rate during this two hour interval. It seems scarcely possible that such continuity of events could be coincident.

The graphs permit an analysis of the individual participation of the stratum basale and the stratum spinosum. The former attains its maximum mitotic response in one hour while the latter required two hours of stimulation. The stratum basale apparently began its recovery phase while the stratum spinosum continued to decline in the mitotic rate at the end of the experiment.

CONCLUSIONS

1. This is believed to be the first work presenting some tangible information on the exact course of epidermal regeneration at rest, and during and after stimulation. The palmar and plantar pads of the cat were used on account of their striking histological similarity to the human palmar and plantar epidermis.
2. In the resting epidermis, the majority of mitoses were found in the lower part of the stratum germinativum, but the lower one-third of the stratum spinosum was more active than the stratum basale.
3. In response to stimulation the peak of the total mitoses was reached in two hours; after which a decline in the mitotic rate took place despite continued stimulation.
4. The stratum basale and the stratum spinosum responded independently. The peak of the regenerative response of the former was reached in one hour after stimulation, while that of the latter occurred after two hours.
5. Neither the stratum basale nor the stratum spinosum was able to maintain "peak production"; once the maximum was attained there occurred a gradual decrease in the rate of mitoses.

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DISCUSSION

Dr. P. Arthur Delaney, *Chicago:* My experience in studying mitotic figures has been somewhat limited, I must confess. I was very much interested in the position of mitotic figures. I do not know just how valuable that would be to the pathologist, however. He sometimes has to look for other things for a criterion for malignancy.

I would be interested in finding out if any abnormal mitoses have been seen away from the basal layer. I wonder if after excessive stimulation you have observed abnormal mitotic figures, whether there was more than normal mitoses, or have you seen direct mitoses?

[Editor's note. Unfortunately this discussion had to be published without author's correction, due to Dr. Delaney's delay in returning the copy submitted to him.]

Dr. Joseph M. Thuringer, *Oklahoma City:* The question of amitoses comes up quite frequently and I must confess that I have never seen amitosis taking place in the epidermis, though binucleate cells are occasionally present. Often cells in late telophase, if cut obliquely, may simulate cells undergoing amitosis. In regard to the other question, this represents a case of stimulation beyond the regenerative capacity of the epidermis, since the results showed that the peak of regeneration had been reached after two hours of exercise, while the stimulation continued an additional three hours.

The utilization of the mitotic index is of value only when based on the study of many sections representing several square millimeters of skin in order to obtain representative values. Our experience has shown that cellular proliferation occurs in localized areas, the so-called growth waves; the inclusion or omission of one or two growth waves would alter a cell count materially. The occurrence of these growth waves in the epidermis should not be considered exceptional, since we know that in other organs, for example the liver and the kidney, not all cells are simultaneously in the same state of activity; some are secreting, some resting, and others regenerating.